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State-of-the-Art Minireview

Effects of Granulocyte-Macrophage Colony-Stimulating Factor on the Levels of VLDL and LDL Receptor mRNAs *in vivo*

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We investigated the mechanism by which granulocyte-macrophage colony-stimulating factor (GM-CSF) lowers plasma cholesterol levels. Recombinant human GM-CSF (rhGM-CSF) was administered to normal and Watanabe heritable hyperlipidemic (WHHL) rabbits. Treatment with rhGM-CSF reduced the levels of cholesterol and triglyceride in these animals. *In vitro* colony assay for hematopoietic progenitors indicated that rhGM-CSF was capable of supporting granulocyte-macrophage colony formation in rabbits, suggesting that rhGM-CSF stimulates macrophage function even in rabbits. Northern blot analysis of rabbit very-low-density lipoprotein (VLDL) receptor showed that rhGM-CSF elevated the levels of VLDL receptor mRNA 2.6- and 1.8-fold in muscles of normal and WHHL rabbits, respectively, 1.5 hours after a single injection. Increases of 1.5- and 1.4-fold were observed in muscles of these rabbits after 5 days of administration. No changes were found in the LDL receptor mRNA levels in liver, spleen or bone marrow. These findings show that the lowering of lipids by GM-CSF may be mediated through the up-regulation of the VLDL receptor mRNA and the enhancement of macrophage function. *J Atheroscler Thromb, 1996; 2: 76-80.*

Key words: GM-CSF, VLDL receptor, cholesterol-lowering

Human granulocyte-macrophage colony-stimulating factor (GM-CSF) has been shown to reduce cholesterol levels in humans (1) although this molecule was originally found to promote the proliferation and maturation of granulocytic and monocytic lineages *in vitro* and *in vivo* (2, 3). This finding suggests that GM-CSF may play an important role in lipid metabolism. However, the mechanism(s) by which GM-CSF lowers plasma cholesterol levels is not well understood.

Macrophage colony-stimulating factor (M-CSF) promotes the proliferation and differentiation of monocytic lineage and enhances the functions of the mature cells (4). In addition, this cytokine also lowers plasma cholesterol levels in animals and humans, the mechanisms of which have been investigated extensively (5-10).

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Our *in vivo* experiment showed that the cholesterol-lowering effect of recombinant human GM-CSF (rhGM-CSF, Hoechst Japan Co Ltd., Tokyo, 11) persisted in normal and cholesterol-fed rabbits even after cessation of the treatment (12). Conversely, Shimano *et al.* reported that plasma cholesterol levels normalized in normal and Watanabe heritable hyperlipidemic (WHHL, 13) rabbits soon after cessation of M-CSF administration (6). Moreover, rhGM-CSF reduced the levels of triglyceride in rabbits, whereas M-CSF did not. According to the different modes of the biological actions of the two molecules, we hypothesized that the effects of these cytokines on the metabolism of lipoprotein might be mediated *via* different pathways.

In this study, we found distinct effects of GM-CSF and M-CSF on the levels of mRNA for lipoprotein receptors in individual organs in rabbits.

Actions of GM-CSF on Mature Hematopoietic Cells and Their Progenitors

rhGM-CSF administration to rabbits did not induce the

increase in levels of circulating granulocytes or monocytes in rabbits (12). Although rhM-CSF lowered cholesterol levels, no change in monocyte count was observed in either group of rabbits treated with rhM-CSF (6). Our previous study showed that a large amount of mouse recombinant GM-CSF was required to increase circulating granulocyte and monocyte levels in a mouse model (14). Comparison of the human and murine GM-CSF cDNA sequences showed a homology of 60% at the DNA level and of 54% at the protein level (2). The rabbit GM-CSF protein has not yet been purified. To determine the biological activity of rhGM-CSF on granulocyte-macrophage progenitor cells in rabbits, rhGM-CSF was therefore added to bone marrow cells from humans and rabbits in a methylcellulose culture system. The numbers of granulocyte-macrophage colonies induced by rhGM-CSF in normal and WHHL rabbits were equivalent to those in humans as shown in Table 1, indicating that the human molecule functions in rabbits as it does in humans. This finding suggests that rhGM-CSF is capable of enhancing macrophage function in rabbits.

Lowering of Plasma Lipid Concentrations

Two-week administration of rhGM-CSF at a dose of 20 μg/kg/day lowered plasma cholesterol and triglyceride levels in normal and cholesterol-fed rabbits (12). A marked reduction in cholesterol was observed in very-low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) levels with a small but significant decrease in high-density lipoprotein cholesterol (HDL-C) levels. The cholesterol-lowering effect of rhGM-CSF persisted for at least 2 weeks following termination of the treatment in the rabbits (12). On the other hand, neither triglyceride nor HDL-C levels were altered in normocholesterolemic rabbits treated with 100 μg/kg/day rhM-CSF for 7 days although the potent cholesterol-lowering effect was observed (6). In addition, plasma cholesterol levels normalized 2 days after termination of rhM-CSF treatment in accordance with the report by Stoudemire *et al.* (6, 7).

Seven-day administration of rhGM-CSF at 20 μg/kg/day reduced total cholesterol levels, but not either triglyceride or HDL-C levels in WHHL rabbits (12). Although the total doses used and the duration of the treatment were different between normal and WHHL rabbits, the major reason why rhGM-CSF administration did not significantly change the levels of triglycerides may be due to a defect in the LDL receptor in WHHL rabbits. This animal model shows the decreased uptake of VLDL and IDL by the liver and clears VLDL and intermediate-density lipoprotein (IDL) from plasma at a markedly delayed rate, giving rise to extremely high levels of LDL-C and triglyceride (15-17). Thus, the accumulation of triglycerides was not significantly altered by rhGM-CSF in WHHL rabbits, although the VLDL receptor mRNA levels were increased in these animals by treatment with rhGM-CSF as described below.

Table 1. CFU-GM-derived colony formation by rhGM-CSF.

	No. of colonies/10^5 cells (mean±SD)
Human (n=5)	128.6±24.9
Normal rabbit (n=5)	121.1±7.2
WHHL rabbit (n=3)	117.3±10.7

Bone marrow cells were obtained from five healthy human volunteers, five normal rabbits and three WHHL rabbits. 10^5 cells were cultured in Iscove's modification of Dulbecco's medium supplemented with 20% fetal calf serum, 100 U/mL penicillin-streptomycin, 0.9% methylcellulose and 200 U/mL rhGM-CSF in 35-mm Petri dishes. Duplicate plates were counted for each sample. Data are expressed as mean±SD.

LDL and VLDL Receptor mRNA Levels

The rabbit VLDL receptor was molecularly cloned by Takahashi *et al.* in 1992 (18). Comparison of the amino acid sequence of rabbit VLDL receptor with that of the rabbit LDL receptor shows striking homology and extensive structural similarity. A critical difference in the mature protein structure between the two receptors is the number of cysteine-rich repeat sequences at the N terminus (15, 18). However, these two lipoprotein receptors have distinct ligand specificities and tissue distributions. The VLDL receptor binds apolipoprotein E (apoE)-containing lipoproteins enriched with triglyceride and its mRNA is abundant in muscle, heart and adipose tissue with little expression in liver. In addition, the human VLDL receptor gene is located on chromosome 9, whereas the human LDL receptor gene is on chromosome 19 (19). These findings suggest that the VLDL receptor plays an important role in the metabolism of apoE-containing lipoproteins enriched with triglyceride and that lipoprotein metabolism is regulated by at least three receptors including LDL receptor, remnant receptor and VLDL receptor.

To determine the mechanism(s) by which GM-CSF lowers cholesterol levels, we examined the effects of rhGM-CSF on the levels of LDL and VLDL receptor mRNAs by Northern blot analysis. rhGM-CSF or human urinary M-CSF (HuM-CSF, the Morinaga Milk Industry Co., Tokyo) was injected into normal and WHHL rabbits, whereas an equal amount of human serum albumin (HSA) was used in controls. The organs from rabbits treated with rhGM-CSF, HuM-CSF or HSA were removed 1.5 hours after the single injection and the 5-day administration, followed by isolation of RNA. In rabbits treated with the single injection, an increased was found in the levels of VLDL receptor mRNA in muscle (2.6-fold increase in normal rabbits), but not in the levels of LDL receptor mRNA in liver, spleen or bone marrow. In contrast, the VLDL mRNA levels were not significantly changed in muscle by treatment with HuM-CSF (12). Figure 1 shows a 1.8-fold increase in the levels of VLDL receptor mRNA in the muscle of WHHL rabbits 1.5 hours after the single injection of

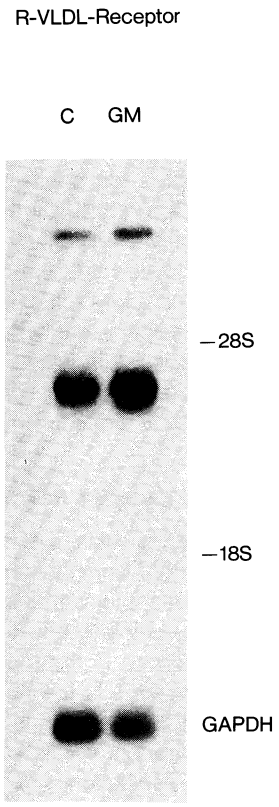

Fig. 1. Effects of rhGM-CSF on the levels of VLDL receptor mRNA in WHHL rabbit muscle. The organs were removed 1.5 hours after the single injection of 20 μg/kg of rhGM-CSF or human serum albumin (HSA, a control solution) and the 5-day administration of 20 μg/kg/day of rhGM-CSF or HSA. Total RNA was isolated from muscle, liver, spleen and bone marrow of the treated rabbits. Two μg of poly(A)+RNA was electrophoresed on an agarose gel (1%), transferred onto a nylon membrane, and hybridized with ^{32}P-labeled probe for rabbit VLDL receptor. The same membrane was rehybridized with a rabbit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe. The positions of the 28S and 18S rRNA subunits are indicated. The levels of the VLDL receptor mRNA in muscle were evaluated in three pairs of WHHL rabbits in both single injection and 5-day administration groups. Results from a representative experiment following the single injection are shown.

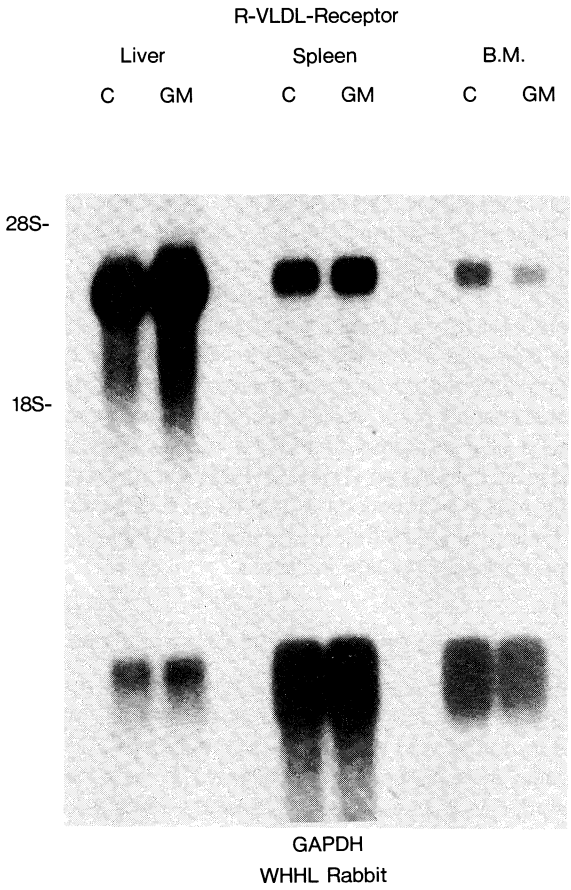

Fig. 2. Effects of rhGM-CSF on mRNA for LDL receptor in WHHL rabbit. The organs from three pairs of WHHL rabbits in the 5-day administration groups were removed 1.5 hours after the final injection of rhGM-CSF or HSA. Two μg of poly (A)+RNA was isolated from liver, spleen and bone marrow and hybridized with ^{32}P-labeled probe for rabbit LDL receptor. The same membrane was rehybridized with the GAPDH probe. The positions of the 28S and 18S rRNA subunits are indicated. Results from a representative experiment are shown.

rhGM-CSF. There were 1.5- and 1.4-fold increases following the 5-day treatment in normal and WHHL rabbits (data not shown), while no changes in the LDL receptor mRNA levels were observed in liver, spleen or bone marrow (Fig. 2). On the other hand, Shimano *et al.* reported increases in LDL receptor mRNA levels in spleen and bone morrow, but not in liver of rabbits treated with rhM-CSF (6). Table 2 summarizes the effects of human GM-CSF and M-CSF on VLDL and LDL receptor mRNA levels in rabbits.

Mechanisms of the Lowering of Triglyceride Levels

Treatment with rhGM-CSF lowered not only cholesterol but also triglyceride levels. The increased uptake of apoE-containing lipoproteins enriched with triglyceride by the up-regulation of VLDL receptor may partially explain the lowering of triglyceride levels by GM-CSF.

Moreover, long-chain acyl-CoA synthetase catalyzes the initial reaction in fatty acid metabolism and this enzyme is primarily distributed in liver, heart and adipose tissue (20). Our preliminary experiment demonstrated that rhGM-CSF

Table 2. Effects of human GM-CSF and M-CSF on the levels of VLDL and LDL receptor mRNAs in rabbits.

	GM-CSF	C-CSF
VLDL receptor (muscle)	↑	→
VLDL receptor (liver)	→	→
LDL receptor (liver)	→	→*
LDL receptor (spleen, bone marrow)	→	↑*

*Effects of human M-CSF on the levels of LDL receptor mRNA in liver, spleen and bone marrow as described in Reference 6.

decreased the levels of acyl-CoA synthetase mRNA as determined by Northern blot analysis with rat long-chain acyl-CoA synthetase cDNA, suggesting that this may be one of the mechanisms responsible for the reduction in triglyceride levels. Further studies are necessary to clarify the role of this enzyme in the effects of treatment with GM-CSF.

Conclusions

Lowering of plasma cholesterol and triglyceride levels was induced by the *in vivo* treatment of rhGM-CSF in normal, cholesterol-fed and WHHL rabbits. We attempted to determine the mechanism(s) and showed that the cholesterol-lowering effect may be mediated through the enhancement of macrophage function and the up-regulation of VLDL receptor mRNA expression in muscle. Our findings also demonstrated distinct effects of GM-CSF and M-CSF on the levels of mRNA for LDL and VLDL receptors. The role of the VLDL receptor in the metabolism of triglyceride-rich lipoproteins in muscle, heart and adipose tissue remains to be elucidated.

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